Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplemental Material for:

Design and synthesis of novel ^{18}F-radiolabelled glucosamine derivatives for cancer imaging

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1. **General experimental procedures, materials and instrumentation**

All reactions were performed under anhydrous conditions and an atmosphere of nitrogen in flame-dried glassware unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H-NMR) homogenous materials.

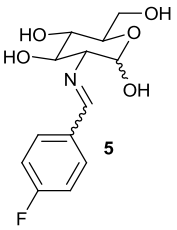
Solvents and reagents: All solvents were purified and dried according to standard methods prior to use. All chemicals were handled in accordance with COSHH regulations. All reagents were used as commercially supplied.

Flash chromatography (FC) was always performed on silica gel (Merck Kieselgel 60 F_{254} 320-400 mesh) according to the method of W. C. Still, unless otherwise stated. Thin Layer Chromatography (TLC) was performed on Merck aluminium-backed plated pre-coated with silica (0.2 mm, 60 F_{254}) which were visualised either by quenching of ultraviolet fluorescence (λ = 254 and 366 nm) or by charring with 10% $KMnO_4$ in 1M H_2SO_4. ^{1}H NMR spectra: These were recorded at 400 MHz on a Bruker AV-400 or on a Bruker AV-500 instrument. Chemical shifts (δ_H) are quoted in parts per million (ppm), referenced to the appropriate residual solvent peak. Coupling constants (*J*) are reported to the nearest 0.5 Hz. ^{13}C NMR spectra: These were recorded at 100 MHz on a Bruker AV-400 instrument. Chemical shifts (δ_C) are quoted in ppm, referenced to the appropriate residual solvent peak. Mass spectra: Low resolution mass spectra (*m/z*) were recorded on either a VG platform II or VG AutoSpec spectrometers, with only molecular ions (M^+, MH^+, MNa^+, MK^+, MNH_4^+) and major peaks being reported with intensities quoted as percentages of the base peak.

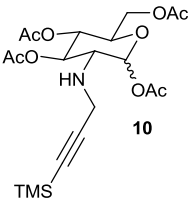
[^{18}F]Fluoride was produced by a cyclotron (GE PETrace) using the $^{18}O(p,n)^{18}F$ nuclear reaction with 16.4 MeV proton irradiation of an enriched [^{18}O]H_2O target.

2. **Synthesis of non-labelled compounds**

Compounds **3**, **4**, **6**, **7** and **12** were synthesised according to literature procedures. [1,2,3]

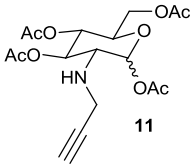


To a stirred solution of 2-(4-fluorobenzilidene)amino-β-1,3,4,6-tetra-*O*-acetyl-D-glucosamine (116 mg, 0.25 mmol) in MeOH/H_2O (5:2, 3 ml) was added K_2CO_3 (140 mg, 1 mmol) and the reaction was stirred for 1 hour.[4-5] The mixture was concentrated *in vacuo* and the resulting residue was purified by flash column chromatography (DCM:MeOH, 9:1) to afford the desired compound as an off-white solid (52 mg, 73 %) as a mixture of *E/Z* 3:1; (Mixture of *E/Z*) δ_H (400 MHz, MeOD-D_4) 3.02 (1H, dd, *J* = 9.3, 7.8 Hz), 3.40-3.49 (2H, m), 3.70 (1H, dd, *J* = 9.8, 8.8 Hz), 3.76 (1H, at, *J* = 5.9 Hz), 3.95 (1H, dd, *J* = 11.7, 2.0 Hz), 4.08 (1H, at, *J* = 9.8 Hz), 5.12 (1/3H, d, *J* = 3.4 Hz); δ_C (100 MHz, MeOD-D_4) 61.5, 70.3, 71.4, 72.1, 74.7, 75.3, 76.8, 78.0, 93.6, 95.5, 115.1 (d, *J* = 22.5 Hz), 115.2 (d, *J* = 22.5 Hz), 130.4, 130.5 (d, *J* = 12.9 Hz), 131.6 (d, *J* = 8.0 Hz), 132.3 (d, *J* = 3.2 Hz), 163.3 (d, *J* = 12.9 Hz), 163.7; δ_F (377 MHz, MeOD-D_4) -105.2, -111.1; *m/z* (ESI) ($[M+H]^+$) *calc.* 286.1085, *found* 286.1080.



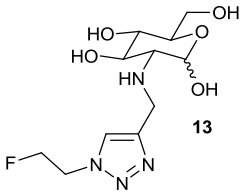
To a stirred solution of 1,3,4,6-*O*-acetyl-D-glucosamine **9** (500 mg, 1.3 mmol) in MeCN/H_2O (3:1, 56 ml) was added 3-(trimethylsilyl)-2-propynal (347 µl, 2.6 mmol) and the resulting mixture was stirred for 30 minutes at room temperature. $NaBH_3CN$ (240 mg, 3.8 mmol) was added and the reaction was stirred for a further 30 minutes, at which point H_2O (25 ml) was added and the reaction mixture was extracted with EtOAc (3 x 25 ml), and the organic layers were collected and washed with brine (25 ml), before being dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (Petrol:EtOAc, 1:1) to afford the desired compound **10** as a viscous yellow oil (575 mg, 90 %, α:β 3:1); δ_H (400 MHz, $CDCl_3$) (α anomer) 0.20 (9H, s), 2.06 (3H, s), 2.11 (3H, s), 2.12 (3H, s), 2.19 (3H, s), 3.10 (1H, dd, *J* = 9.8, 8.3 Hz), 3.49 (2H, d, *J* = 5.9 Hz), 3.78-3.83 (1H, m), 4.10 (1H, dd, *J* = 12.2, 2.5 Hz), 5.08-5.14 (2H, m), 5.54 (1H, d, *J* = 8.3 Hz); (β anomer) 0.21 (9H, s), 2.05 (3H, s), 2.08 (3H, s), 2.10 (3H, s), 2.15 (3H, s), 3.18 (1H, dd, *J* = 10.3, 8.8 Hz), 3.64 (2H,

as), 3.75-3.79 (1H, m), 4.09 (1H, dd, *J* = 12.7, 2.0 Hz), 5.27 (2H, dd, *J* = 10.8, 9.3 Hz), 5.88 (1H, d, *J* = 8.8 Hz); δ_C (100 MHz, $CDCl_3$) (α and β anomers combined) -0.2, 0.2, 20.7, 20.8, 20.9, 21.2, 36.3, 51.7, 59.5, 61.8, 63.7, 68.3, 68.7, 72.2, 72.5, 73.9, 88.1, 89.3, 95.4, 104.1, 168.9, 169.7, 170.7, 171.2; *m/z* (ESI) ($[M+H]^+$) *calc.* 458.1841, *found* 458.1845.



11

To a stirred solution of compound **10** (570 mg, 1.16 mmol) in THF (25 ml) was added TBAF (1M in THF, 3.5 ml) and the resulting mixture was stirred for 1 hour at room temperature (NB - the solution turned dark brown within minutes of addition of TBAF). H_2O (25 ml) was added and the aqueous layer was extracted with EtOAc (3 x 25 ml), and the resulting organic layer was washed with brine (25 ml). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*, before the residue was purified by flash column chromatography (Petrol:EtOAc, 1:1) to afford the desired compound **11** as a yellow viscous oil (127 mg, 28 %); δ_H (400 MHz, $CDCl_3$) 2.06 (3H, s), 2.08 (3H, s), 2.10 (3H, s), 2.15 (3H, s), 2.23 (1H, t, *J* = 2.0 Hz), 3.17 (1H, dd, *J* = 10.8, 9.3 Hz), 3.66 (2H, at, *J* = 2.9 Hz), 3.78 (1H, ddd, *J* = 9.8, 4.4, 2.0 Hz), 4.08 (1H, dd, *J* = 12.2, 2.0 Hz), 4.34 (1H, dd, *J* = 12.2, 4.4 Hz), 5.17 (2H, ddd, *J* = 38.6, 10.8, 8.8 Hz), 5.85 (1H, d, *J* = 9.3 Hz); δ_C (100 MHz, $CDCl_3$) 20.7, 20.8, 20.9, 21.5, 39.7, 61.7, 63.8, 68.5, 70.2, 72.3, 72.6, 79.8, 92.5, 168.5, 169.6, 170.4, 170.7; *m/z* (ESI) ($[M+H]^+$) *calc*. 386.1446, *found* 386.1440.

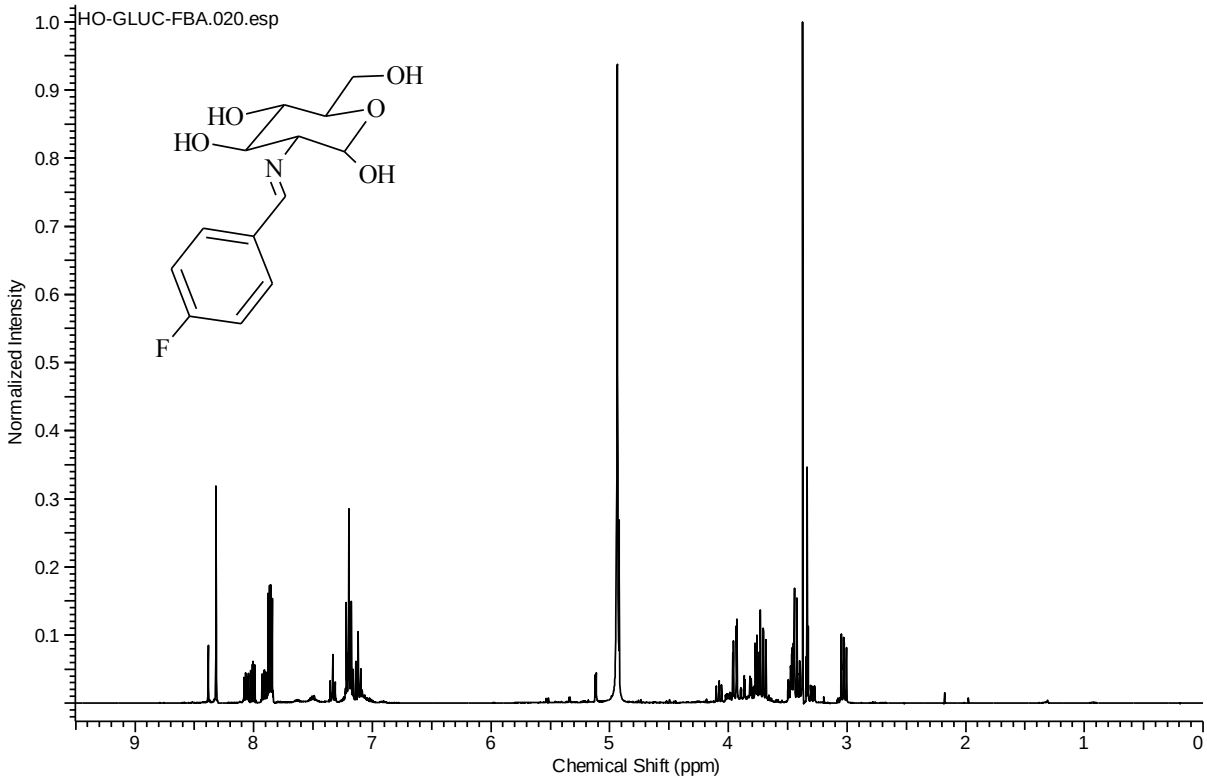


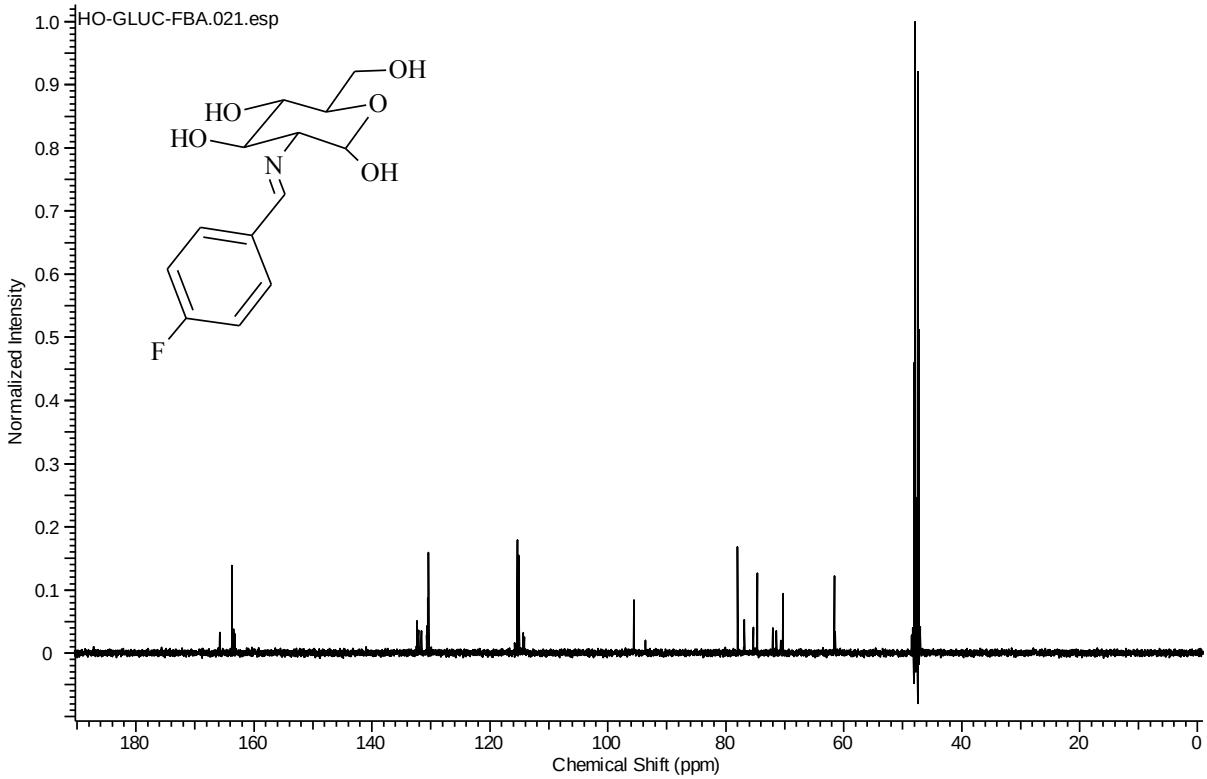
13

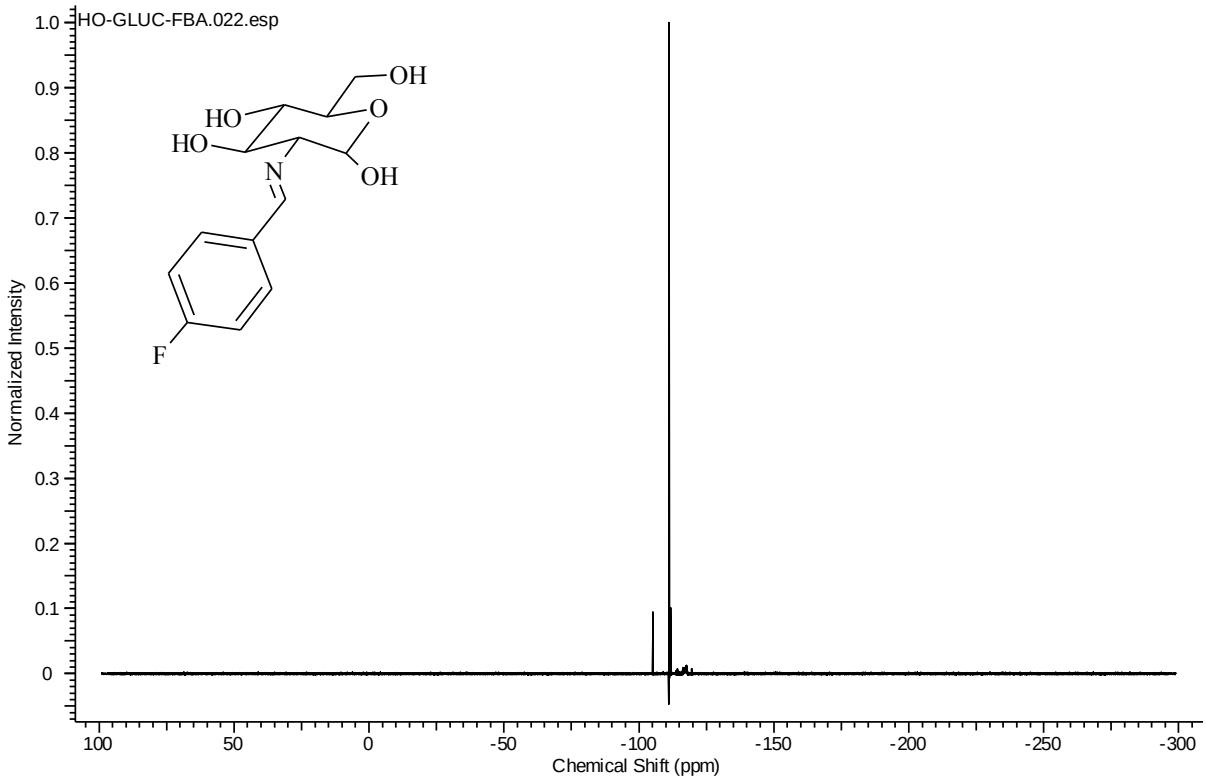
To a stirred solution of compound **11** (100 mg, 0.24 mmol) in DMF (2 ml) was added $CuSO_4$ (1M, 20 µl) and sodium ascorbate (1M, 20 µl), and then fluoroethylazide **12** (0.14 M, 2 ml) and the reaction mixture was stirred for 16 hours at room temperature. EtOAc (15 ml) was added, and the aqueous layer was extracted with further washings of EtOAc (2 x 15 ml). The combined organic layers were washed with brine (15 ml), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was re-dissolved in MeOH (2 ml) and NaOMe (52 mg, 0.96 mmol) was added. The reaction mixture was stirred for 1 hour at room temperature, before being concentrated *in vacuo*. The crude residue was passed through a silica pad (DCM:MeOH, 1:1), before being re-dissolved in DCM (10 ml) and

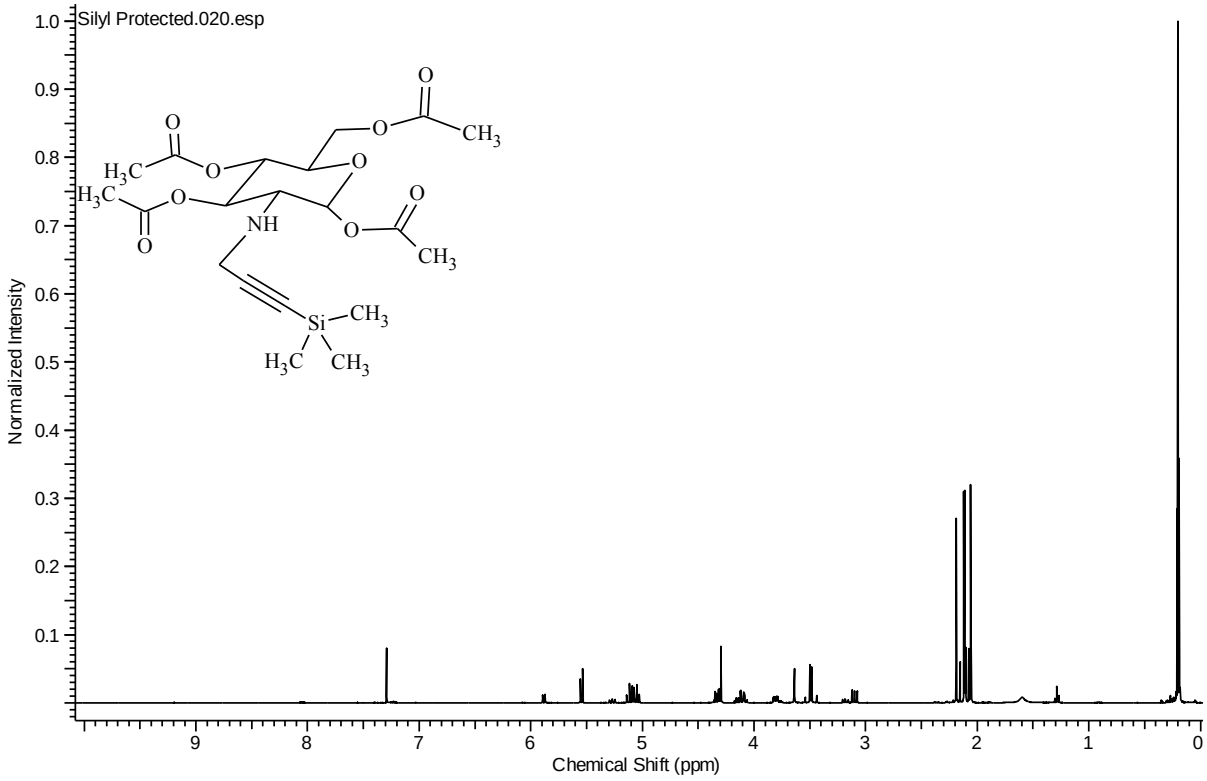
filtered to remove silica, affording the desired compound **13** (31 mg, 42 %) as a yellow solid; δ_H (400 MHz, $CDCl_3$) 3.15 (1H, at, *J* = 10.8 Hz), 3.74-3.84 (1H, m), 4.08 (1H, dm, *J* = 12.2 Hz) 4.31 (1H, dd, *J* = 12.2, 4.4 Hz), 4.67 (2H, dt, *J* = 27.4, 4.9 Hz), 4.81 (2H, dt, *J* = 46.9, 4.4 Hz), 5.06 (1H, at, *J* = 9.8 Hz), 5.42 (1H, at, *J* = 9.8 Hz), 5.84 (1H, d, *J* = 8.8 Hz), 8.05 (1H, s); δ_F (377 MHz, MeOD-D_4) -221.6; *m/z* (ESI) ($[M+H]^+$) *calc.* 307.1412, *found* 307.1412.

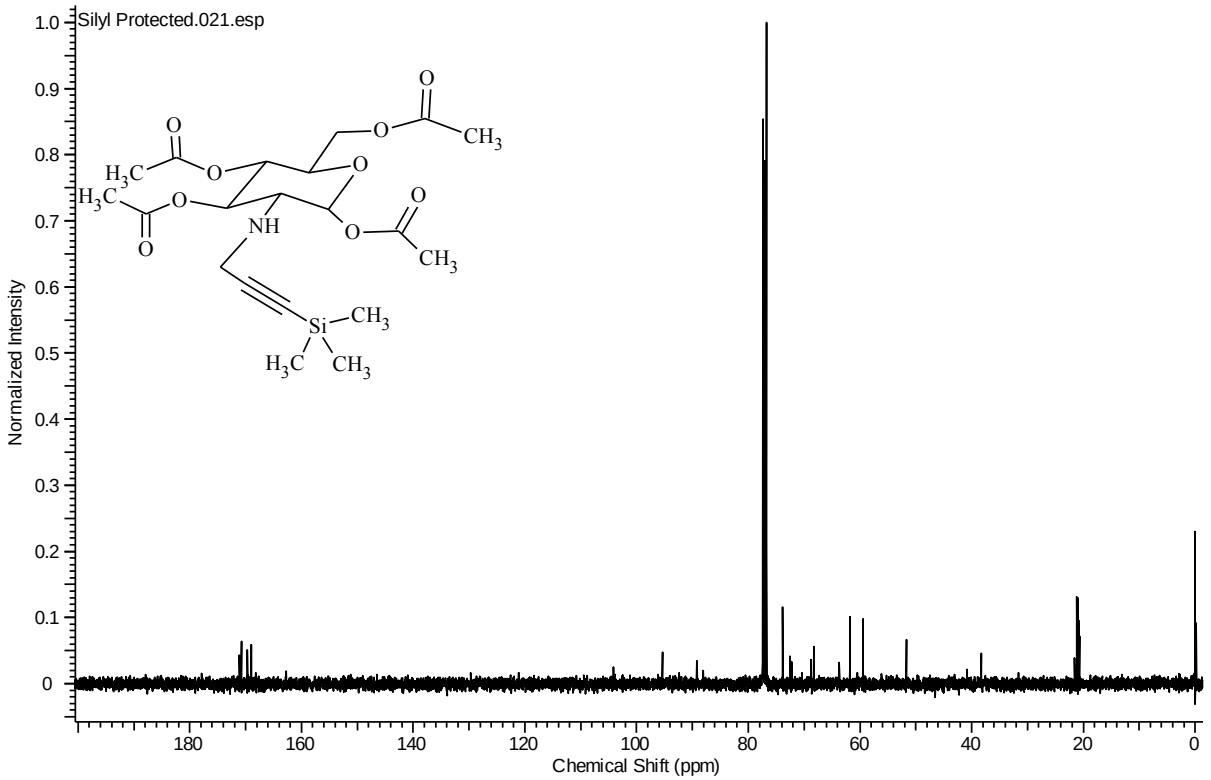
3. **NMR Spectra of relevant compounds**

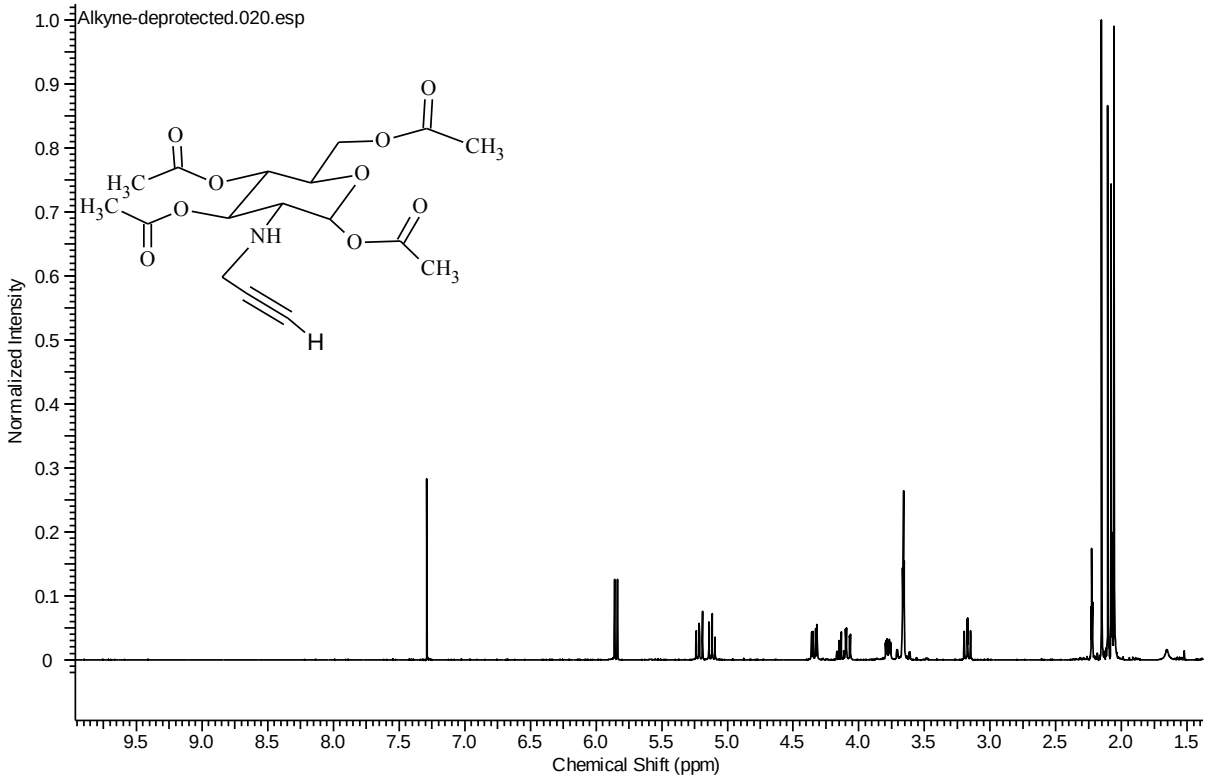


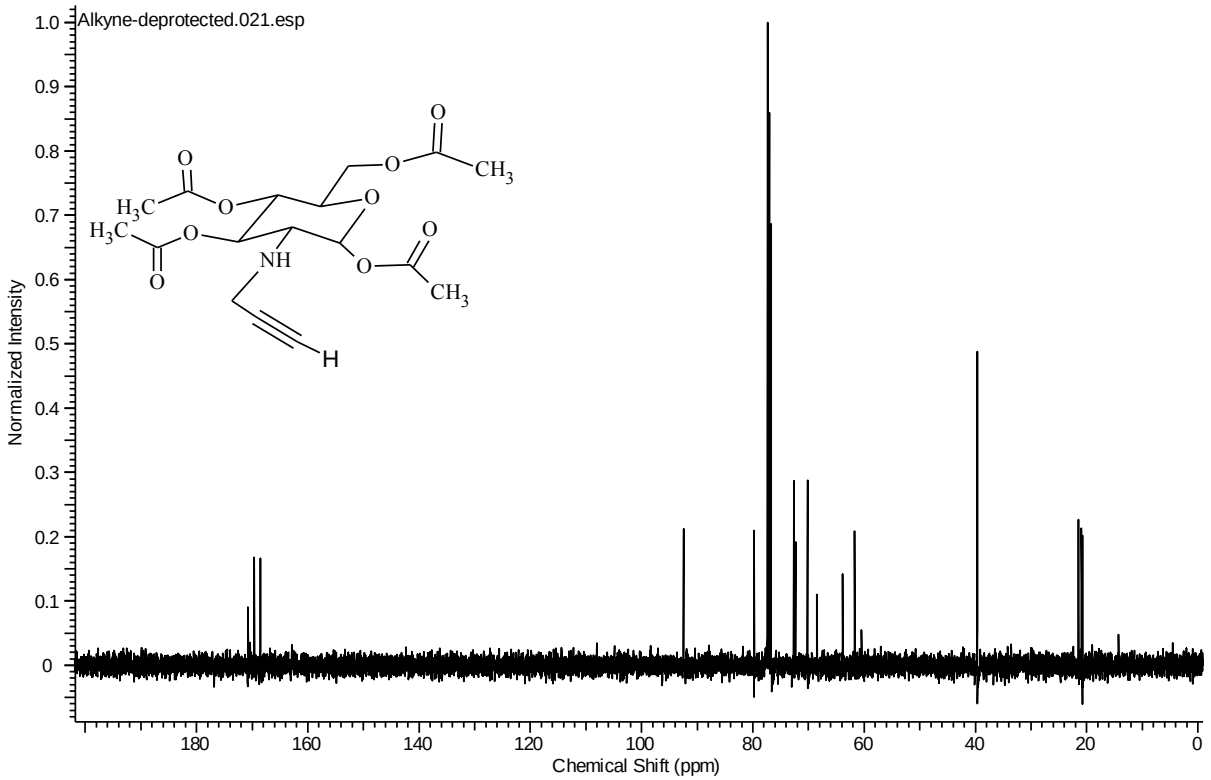


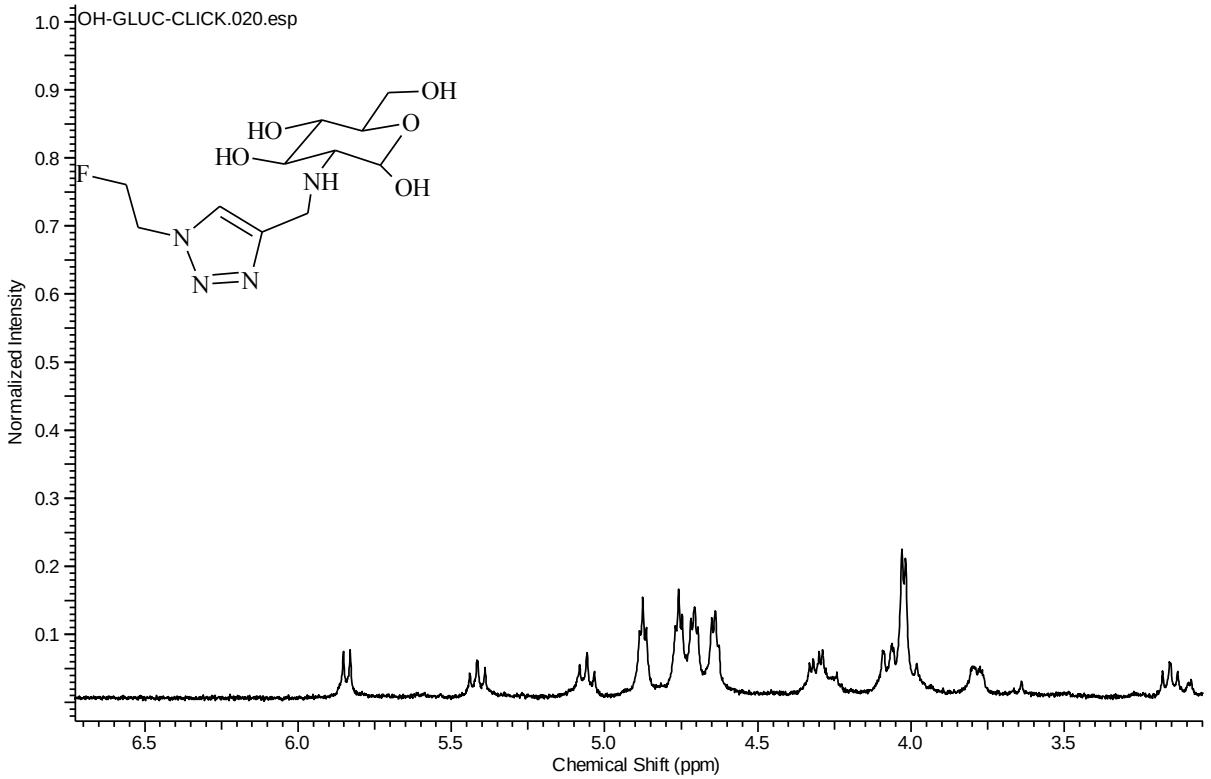


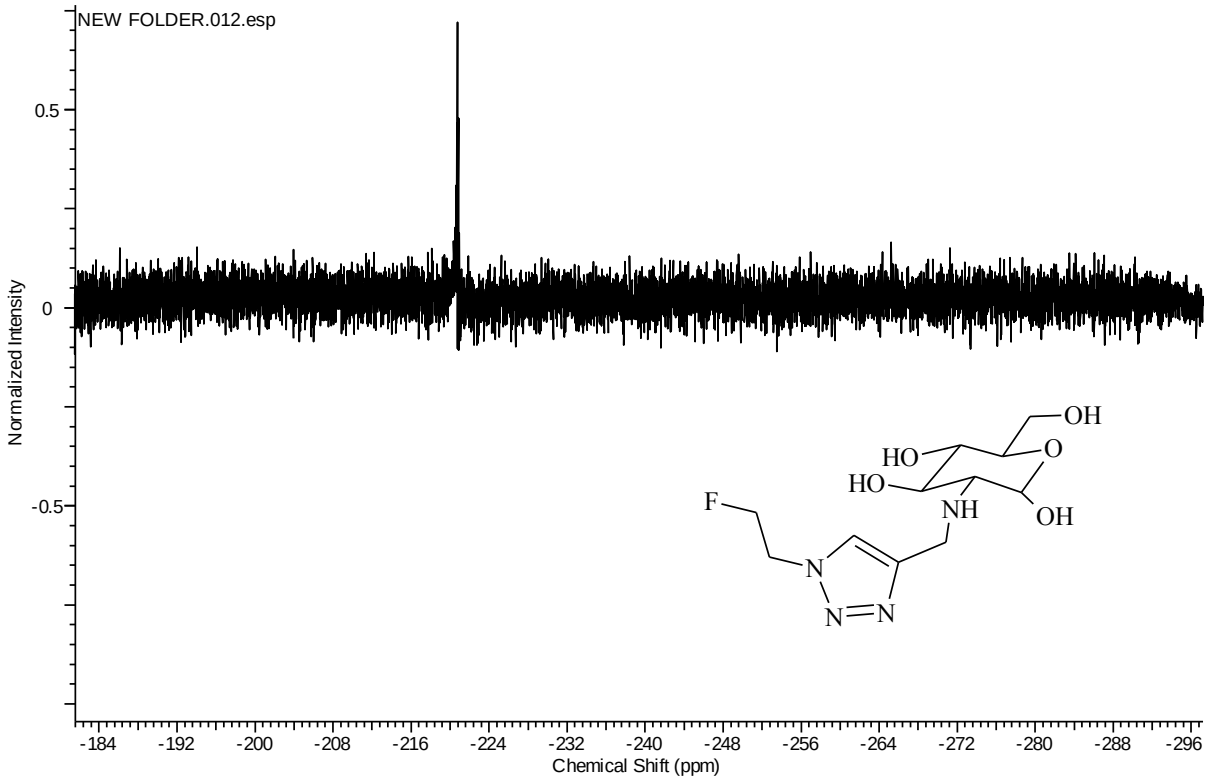




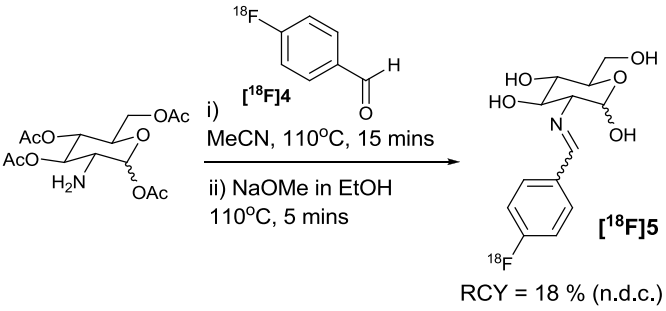




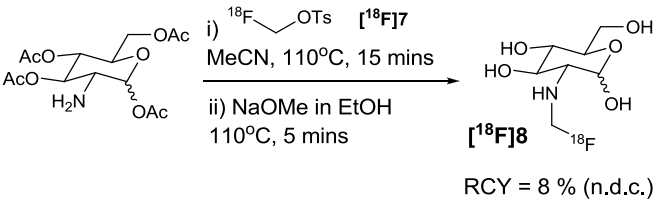



NEW FOLDER.012.esp
0.5
0
-0.5
Normalized Intensity
-184
-192
-200
-208
-216
-224
-232
-240
-248
-256
-264
-272
-280
-288
-296
Chemical Shift (ppm)
OH
HO
O
HO
NH
OH
F
N
N
N

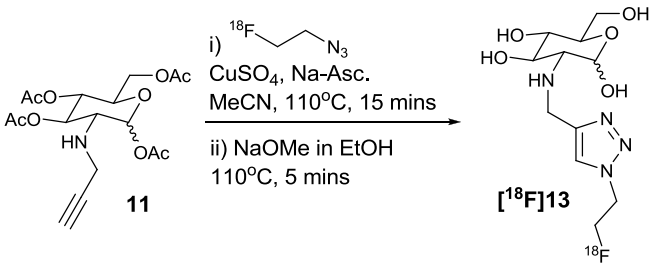
4. ^{18}F Radiosynthesis of compounds 5, 8 and 13



[^{18}F]Fluorobenzaldehyde **[^{18}F]4** was prepared in accordance with a previously published method.[1] **[^{18}F]5** was prepared by reacting 1,3,4,6-tetra-*O*-acetylglucoseamine (5 mg in 300 µl MeCN) with purified **[^{18}F]4** (50-150 MBq in 500µl MeCN) at 110^{o}C for 15 minutes. After subsequent heating of the reaction mixture with sodium methoxide (2 mg in 50 µl MeOH) for 5 minutes, the reaction mixture was diluted with H_2O (upto 1 mL) and injected into a semi-preparative column (Luna 5u NH_2 100A [Phenomonex]; mobile phase ethanol:water isocratic (1:4); flow rate 3 mL/min). The fractions between 9 and 10 minutes were collected and evaporated to dryness, and reformulated in saline (0.3-0.5 mL). Total synthesis time = 95 minutes.



[^{18}F]Fluoromethyltosylate **[^{18}F]7** was prepared in accordance with a previously published method.[3] **[^{18}F]7** was prepared by reacting 1,3,4,6-tetra-*O*-acetylglucoseamine (5 mg in 300 µl MeCN) with purified **[^{18}F]7** (100-200 MBq in 3 % H_2O in MeCN) at 110^{o}C for 15 minutes. After subsequent heating of the reaction mixture with sodium methoxide (2 mg in 50 µl MeOH) for 5 minutes, the reaction mixture was diluted with H_2O (upto 1 mL) and injected into a semi-preparative column (Luna 5u NH_2 100A [Phenomonex]; mobile phase ethanol:water isocratic (1:4); flow rate 3 mL/min). The fractions between 4 and 6 minutes were collected and evaporated to dryness, and reformulated in saline (0.3-0.5 mL). Total synthesis time = 95 minutes.

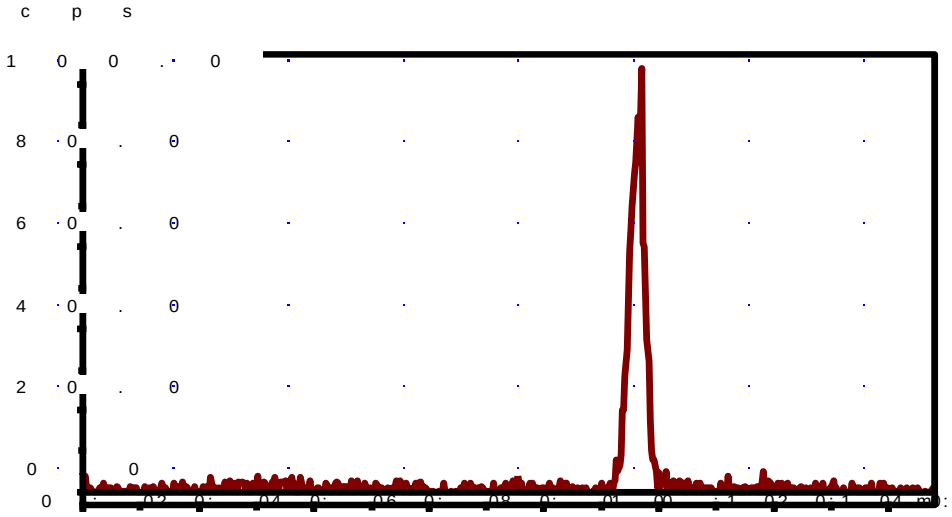


RCY = 15 % (n.d.c.)

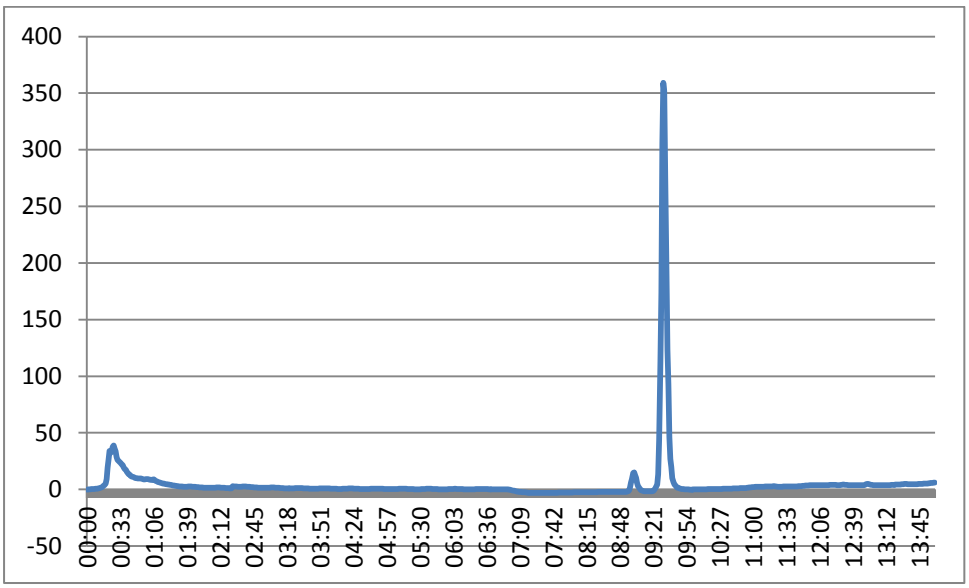
[^{18}F]2-Fluoroethylazide **[^{18}F]12** was prepared in accordance with a previously published method.[1] **[^{18}F]13** was prepared by reacting alkyne precursor **11** (2 mg in 300 μL MeCN) with distilled **[^{18}F]12** (250-370 MBq in 100 μL MeCN) in the presence of $CuSO_4$ (0.25 M, 25 μL) and sodium ascorbate (0.25M, 25 μL) at 110°C for 15 minutes. After subsequent heating of the reaction mixture with sodium methoxide (2 mg in 50 μl MeOH) for 5 minutes, the reaction mixture was diluted with H_2O (upto 1 mL) and injected into a semi-preparative column (Luna 5u NH_2 100A [Phenomonex]; mobile phase MeCN:H_2O isocratic (95:5); flow rate 3 mL/min). The fractions at 6 minutes was collected and evaporated to dryness, and reformulated in saline (0.3-0.5 mL). Total synthesis time = 100 minutes.

4. **Analytical ^{18}F Radiosynthesis HPLC traces**

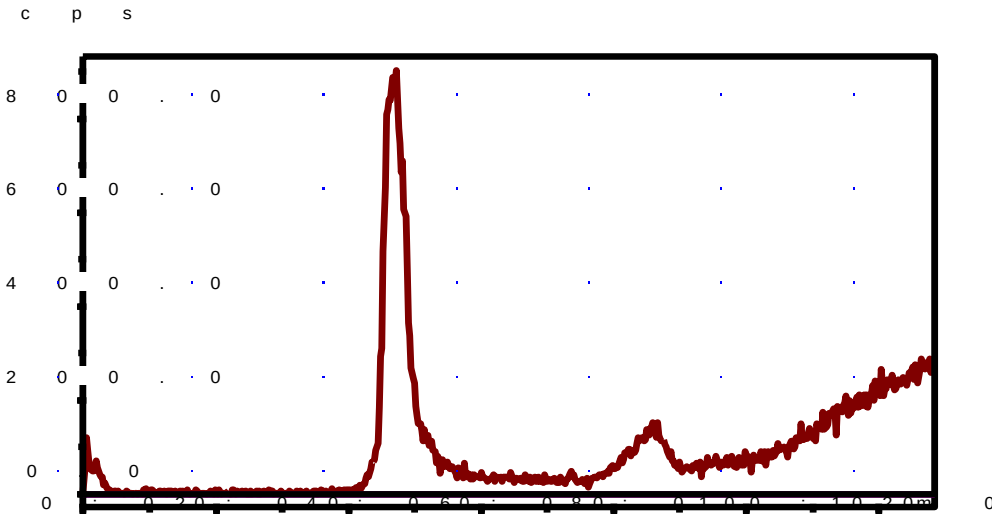
Radio-HPLC trace of **[^{18}F]5**



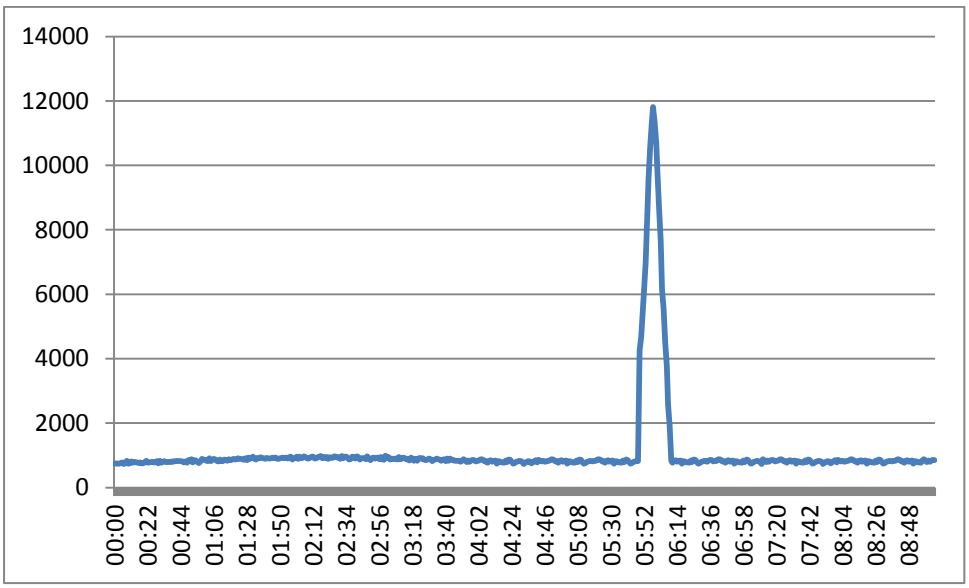
UV-HPLC trace of **[^{19}F]5**



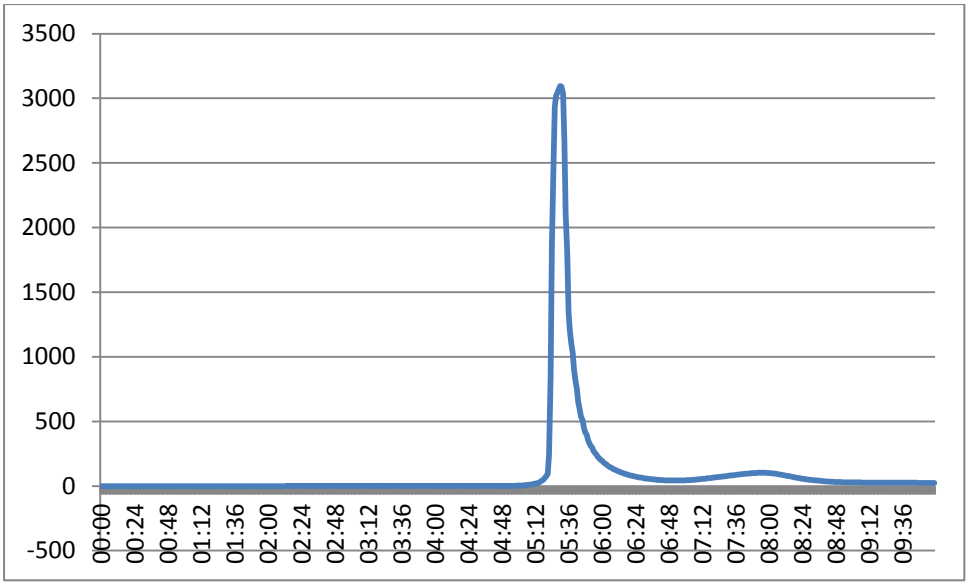
Radio-HPLC trace of **[^{18}F]8**



Radio-HPLC trace of **[^{18}F]13**



UV-HPLC Trace of **[^{19}F]13**



4. **Biological procedures**

Biodistribution studies

[^{18}F]13 and [^{18}F]FDG (~3.7 MBq) were each injected via the tail vein of anaesthetized tumor-bearing BALB/c nude mice. The mice were maintained under anesthesia and sacrificed by exsanguination via cardiac puncture at 60 min post radiotracer injection to obtain blood, plasma, heart, lung, liver, stomach, intestine, spleen, kidney, muscle, bone, brain, urine and tumor. Tissue radioactivity was determined on a gamma counter (Cobra II Auto-Gamma counter, Packard Biosciences Co, Pangbourne, UK) and decay corrected. Data were expressed as percent injected dose per gram of tissue.

***In vivo* tumor models**

All animal experiments were performed by licensed investigators in accordance with the United Kingdom Home Office Guidance on the Operation of the Animal (Scientific Procedures) Act 1986 and within the newly-published guidelines for the welfare and use of animals in cancer research. Female BALB/c nude mice (aged 6 - 8 weeks; Charles River, Wilmington, MA, USA) were used. Tumor cells (2 x 10^6) were injected subcutaneously on the back of mice and animals were used when the xenografts reached ~ 100 mm^3. Tumor dimensions were measured continuously using a caliper and tumour volumes were calculated by the equation: volume = (π / 6) × a × b × c, where a, b, and c represent three orthogonal axes of the tumor.

PET imaging studies

Dynamic [^{18}**F]13** imaging scans were carried out on a dedicated small animal PET scanner (Siemens Inveon PET module, Siemens Medical Solutions USA, Inc., Malvern, PA, USA) following a bolus *i.v.* injection of ~3.7 MBq of the radiotracer into tumor-bearing mice.[3] Dynamic scans were acquired in list mode format over 60 min. The acquired data were then sorted into 0.5 mm sinogram bins and 19 time frames for image reconstruction (4 × 15 s, 4 × 60 s, and 11 × 300 s), which was done by filtered back projection. The Siemens Inveon Research Workplace software was used for visualization of radiotracer uptake in the tumor; 30 to 60 min cumulative images of the dynamic data were employed to define 3-dimensional (3D) regions of interest (ROIs). The count densities were averaged for all ROIs at each time point to obtain a time versus radioactivity curve (TAC). Tumor TACs were normalized to injected dose, measured by a VDC-304 dose calibrator (Veenstra Instruments, Joure, The Netherlands), and expressed as percentage injected dose per mL tissue. For image visualization, iterative reconstruction was performed (3D-OSEM).

6. References

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